Antibiotic resistance pattern of urinary isolates in a Rural Medical College of Maharashtra

Sanjaykumar More¹, Sourabh Chakraborty²*, Sandeep L. Nilekar³, Deepali M. Kulkarni⁴, Rajesh S. Ovhal⁵

¹Professor & HOD, ²Resident, ³,⁴Associate Professor, ⁵Assistant Professor, Dept. of Microbiology,

*Corresponding Author:
Email: dr.sourabchakraborty@yahoo.in

Abstract
Objective: To determine resistance pattern of urinary isolates in a rural medical college of Maharashtra.
Methods: Urine samples were collected using midstream clean catch method in clinically diagnosed UTI cases from August 2016 to February 2017. A total of 320 samples were analyzed under supervision of qualified microbiologist. Antibiotic sensitivity of isolated microorganisms were tested for commonly used antibiotics by Kirby Bauer technique.
Results: Out of 320 samples 200 were positive. The pathogens isolated were E.coli 60 (30%), Pseudomonas 45 (22.5%), Methicillin resistant Staphylococcus aureus 35 (17.5%) & Klebsiella 30 (15%). E.coli being the most common isolate. The isolates showed high degree resistance for Amoxycillin & Cotrimoxazole.
Conclusion: 62.5% of samples showed significant growth. Moderate yield suggest moderate clinical correlations in suspected cases of UTI. E.coli showed low level resistance to Nitrofurantoin (1.67%), Amikacin (3.34%), Norfloxacin (13.3%) & high degree resistance to Cotrimoxazole (63.34%), Piperacillin + Tazobactam (50%), fluoroquinolones (27.04%). It’s very clear from the study E.coli is the MOC infecting uropathogen & developing resistance against the commonly used antibiotics.

Keywords: Uropathogens, Antibiotic resistance, Urinary tract infections.

Introduction
Urinary tract infection is not only common nosocomial infection but an important source of morbidity in community as well.¹,² It is the most frequent cause of illness in human after respiratory infections.³ Neonates, girls, young women and older men are most susceptible to UTI.⁴

Urinalysis only indicates presence of bacteria and leucocytes in the urine, which is indirect evidence of UTI but it can only be confirmed on the basis of microscopy and microbial culture.⁵ In most instances, growth of more than 10⁵ organisms per milliliter from a properly collected midstream "clean-catch" urine sample indicates infection. However, significant bacteriuria is lacking in some cases of true UTI, especially in symptomatic patients, a smaller number of bacteria (10² to 10⁴/mL) may signify infection. In urine specimens obtained by suprapubic aspiration or "in-and-out" catheterization and in samples from a patient with an indwelling catheter, colony counts of 10² to 10⁵/mL generally indicate infection. Conversely, colony counts of >10⁵ /mL of midstream urine are occasionally due to specimen contamination, which is especially likely when multiple species are found.⁶

The vast majority of uncomplicated UTIs are caused by the Gram-negative bacillus Escherichia coli, with other pathogens including Enterococci, Staphylococcus saprophyticus, Klebsiella spp. and Proteus mirabilis.⁷ The extensive and inappropriate use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which, in recent years, has become a major problem worldwide.⁸ Various virulence factors in uropathogens including adhesions, hemolysins, capsular polysaccharides and drug resistance are major decisive factors in development and treatment of infections.⁹

In patients with suspected UTI, antibiotic treatment is usually started empirically, before urine culture results are available. To ensure appropriate treatment, knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory.¹⁰ There are various reports available about changing pattern of pathogen and their susceptibility to routinely used antibiotics in last two decades due to extra chromosomal genetic elements, which simultaneously carry gene for resistance to number of antibiotics and this has made the situation miserable, especially in gram negative bacteria.¹¹

So the databank about antibiotic resistance has to be updated regularly, to achieve maximal clinical response before the sensitivity report gets available and also extensive and inappropriate use of antibiotics can be avoided.

The study is aimed to determine pattern of pathogens isolated, their resistance and sensitivity pattern to different antibiotics in a rural govt. medical college of Maharashtra, so the clinicians can start more appropriate treatment empirically.

Materials and Method
This study was conducted in department of Microbiology, in a rural govt. medical college & hospital of Maharashtra from August 2016 to February 2017. A total of 320 samples were analyzed of patients who were suspected to be having UTI, with prior permission from Institutional Ethics Committee. Samples were collected from clinically diagnosed UTI cases (fever, dysuria, increased frequency of urination).
Specimen were collected by standard “clean catch” mid stream method in patients who had no catheter in place, while in catherized patients, sample was collected in sterile, screw capped, wide mouth container after clamping the catheter for 30 mins. Before collecting the sample, male subjects were asked to clean the genital parts with soap and water and in case of female, they were asked to wash vulva & to separate carefully the labia prior to voiding the urine in sterile bottle. Samples were examined and processed on the Blood agar and McConkey’s agar medium by standard loop method and incubated for atleast 24 hours at 37degree. Plates were observed for bacterial growth.\textsuperscript{12}

Uropathogens were identified on the basis of Gram’s reaction, colony morphology and standard biochemical tests. Antibiotic susceptibility test was carried out for bacterial isolates by Kirby Baur disc diffusion technique. Antibiotics against which sensitivity was tested in the present study included Amoxycillin, Amoxiclav, Ciprofloxacin, Norfloxacin, Cotrimoxazole, Gentamicin, Amikacin, Nitrofurantoin, Cefazidime, Meropenem, Piperacillin + Tazobactam.\textsuperscript{13}

### Table 1: Antibiotic Resistance and Sensitivity Pattern of Isolated Uropathogens

<table>
<thead>
<tr>
<th>E.coli (60)</th>
<th>K.pneumoniae (30)</th>
<th>P.aeruginosa (45)</th>
<th>MRSA (35)</th>
<th>C.freundii (15)</th>
<th>C.koserii (10)</th>
<th>Enterobacter (5)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>AMX</td>
<td>13</td>
<td>24</td>
<td>3</td>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>18</td>
</tr>
<tr>
<td>AMX + Clav</td>
<td>19</td>
<td>26</td>
<td>4</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>21</td>
</tr>
<tr>
<td>COT</td>
<td>22</td>
<td>25</td>
<td>3</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>19</td>
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<tr>
<td>GEN</td>
<td>45</td>
<td>26</td>
<td>1</td>
<td>10</td>
<td>ND</td>
<td>ND</td>
<td>12</td>
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<tr>
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<td>57</td>
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<td>1</td>
<td>14</td>
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<td>23</td>
<td>36</td>
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<td>11</td>
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</table>

**Result**

A total of 320 urine Samples were analyzed in the present study. Out of 320 samples, 200 samples were positive. Most common organism isolated were gram negative organisms. E.coli (30%), Pseudomonas (22.5%), followed by MRSA (17.5%) and Klebsiella (15%). The antibiotics were chosen as per the CLSI guidelines. E.coli showed low level resistance for Nitrofurantoin (1.67%), Amikacin (3.34%), Norfloxacin (13.33%) with high resistance pattern for Cotrimoxazole (63.33%), Fluoroquinolones (35.83%), Piperacillin & Tazobactam (50%). Pseudomonas showed low resistance to Ceftazidime (11.11%), Meropenem (13.33%), Norfloxacin (15.56%). MRSA was highly resistant to Cotrimoxazole (45.71%), Amoxycillin (48.58%), whereas excellent sensitivity to Nitrofurantoin (97.14%). Klebsiella was less resistant for meropenem (6.67%), Norfloxacin (16.67%), Amikacin (13.33%), whereas highly resistant for Cotrimoxazole (83.33%), Amoxycillin (80%), Ceftazidime (60%).

The isolates showed high degree of resistance for Amoxycillin and Cotrimoxazole (Table 1).
Discussion

UTI is a major health problem worldwide & pattern of antibiotic resistance varies in different regions. Injudicial usage of higher antibiotics at community level, making the situation more alarming. In community and hospital setting the etiology of UTI and antimicrobial susceptibility of bacteria causing UTI have been changing over the years.\(^{14,15}\)

Looking at the result the most common organism isolated in our hospital is E.coli (30%), this is consistent with other studies Stamm WE et al (2002), Biswas D et al (2006) in which E.coli is the most common pathogen isolated from patients with UTI.\(^{6,16}\)

Following E.coli our study shows Pseudomonas (22.5%), MRSA (17.5%), Klebsiella (15%) as the common isolates. Our study is in contrast with a study by Jai Pal Paryani et al (2012) showing klebsiella (11.31%), Enterobacter (11.31%), Proteus (7.86%) as the common isolates other than E.coli (64.41%\(^{17}\)).

Enterobacteriaceae having several factors for their attachment to urothelium. The Gram negative bacilli bacteria colonize the urogenital mucosa with adhesion, pili, fimbiae, PI blood group phenotype receptor.\(^{18}\)

E.coli found to be resistant to the 1\(^{st}\) line antibiotics like: Amoxygenillin (78.34%), Amoxygenillin & Clavulanic acid (68.33%), Cotrimoxazole (63.33%). This is in accordance with Srinivasa H et al (1999) stating about resistance of microorganisms to conventional antibiotics.\(^{20}\)

Pseudomonas aeruginosa which is the most common cause of hospital acquired UTI, was less resistant to Fluoroquinolones (17.78%) and cephalosporine (ceftazidime 11.1%) than Aminoglycoside (Amikacin 31.1%), in contrast to a study by Jai Pal Paryani et al., 2012.\(^{17}\)

MRSA is the 3\(^{rd}\) most common isolate in our study, similar to studies by shalini et al., 2011\(^{19}\) and molay Banerjee et al., 2014\(^{21}\) where staph is the 4\(^{th}\) most common isolate. For MRSA resistance pattern is much better than GNB’s.

In our study mean resistance for Cotrimoxazole, amoxicillin & Clavulanic acid, Cephalosporine (ceftazidime), Quinolones (ciprofloxacin & Levofloxacin), were 62.06%, 46.23%, 44.07, 27.29 respectively. From this study it can be seen that Amoxicillin & Clavulanic acid and Cotrimoxazole are nearly having no role against uropathogens due to high degree of resistance. Least resistance pattern were shown by Nitrofurantoin (13.54%), Meropenem (15%), Norfloxacin (19.57%).

Our study suggest Nitrofurantion (13.54% resistance) and Norfloxacin (19.57% resistance) as the 1\(^{st}\) line drug before culture and sensitivity report is available. Both are active against E.coli, klebsiella, MRSA. Both are cost effective and readily available. Nitrofurantoin is better in pregnancy than Norfloxacin,\(^{22}\) Recent study in India showed Nitrofurantoin having best in-vitro susceptibility profile against E.coli. The minimal resistance of Nitrofurantoin against E.coli (1.67%) may be influenced by Nitrofurantoin’s narrow distribution and limited interaction with pathogens outside urinary tract.

In a recent study by Bours PH et al (2010) 29.5% of E.coli was suspected to produce ESBL and, Amikacin and Nitrofurantoin were the only drugs to which > 90% of E.coli was susceptible.\(^{24}\) In the present study Nitrofurantoin is resistant against 1.7% isolates of E.coli, 2.86% isolates of MRSA, 23.3% isolates of klebsiella compared to Nitrofuracin resistant to 13.3%, 31.4%, 13.3% respectively but with an advantage of activity against pseudomomas (15.6% resistance).

Conclusion

In the present study of all samples 62.5% showed significant growth. Moderate yield of positive culture suggest not so good clinical correlation in suspected cases of UTI. But it is certain that due to the rising trend of antibiotic resistance, choice of antibiotics getting lesser day by day. Cotrimoxazole and Ampicillin which was drug of choice previously, now rarely prescribed as empirical therapy. But Norfloxacin and Nitrofurantoin are good alternative to start with antibiotic therapy for ESBL producing E.coli.

References

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